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Published
With international search report.

(54) Title: DNA SEQUENCE CODING FOR A MAMMALIAN GLUCURONYL C5-EPIMERASE AND A PROCESS FOR ITS PRODUCTION

(57) Abstract

An isolated or recombinant DNA sequence coding for a mammalian, including human, glucuronyl C5-epimerase or a functional derivative thereof capable of converting D-glucuronic acid (GlcA) to L-iduronic acid (IdoA); a recombinant expression vector comprising such DNA sequence; a host cell transformed with such recombinant expression vector; a process for the manufacture of a glucuronyl C5-epimerase or functional derivative thereof capable of converting GlcA to IdoA, comprising cultivation of a cell-line transformed with such recombinant expression vector; and a glucuronyl C5-epimerase or functional derivative thereof prepared by such process.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/00703

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C12N 9/90
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	The Journal of Biological Chemistry, Volume 269, No 43, October 1994, Patrick Campbell et al, "Biosynthesis of Heparin/Heparan Sulfate", page 26953 - page 26958 --	1-8
A	WO 9614425 A1 (INALCO S.P.A.), 17 May 1996 (17.05.96) -- --------	1-8

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 June 1998	02 -07- 1998
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Yvonne Siösteen Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

09/06/98

International application No.
PCT/SE 98/00703

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9614425 A1	17/05/96	AU 3926195 A	31/05/96
		CA 2204366 A	17/05/96
		EP 0789777 A	20/08/97
		IT 1271057 B	26/05/97
		IT MI942240 D	00/00/00

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
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ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing: 29 October 1998 (29.10.98)	
International application No.: PCT/SE98/00703	Applicant's or agent's file reference: 2988293
International filing date: 17 April 1998 (17.04.98)	Priority date: 18 April 1997 (18.04.97)
Applicant: LINDAHL, Ulf et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International preliminary Examining Authority on:

01 October 1998 (01.10.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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Facsimile No.: (41-22) 740.14.35

Authorized officer:

J. Zahra

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2988293	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/SE98/00703	International filing date *(day/month/year)* 17.04.1998	Priority date *(day/month/year)* 18.04.1997
International Patent Classification (IPC) or national classification and IPC6 C 12 N 9/90		
Applicant Lindahl, Ulf et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.10.1998	30.06.1999
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88 Telex 17978 PATOREG-S	Authorized officer Yvonne Siösteen/Els Telephone No. 08-782 25 00

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE98/00703

I Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

☐ the international application as originally filed.

☒ the description, pages 1-18, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____.

☒ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-8, filed with the letter of 25.05.1999,
Nos. _____, filed with the letter of _____.

☒ the drawings, ~~sheets~~/fig 1-3, as originally filed,
~~sheets~~/fig _____, filed with the demand
~~sheets~~/fig _____, filed with the letter of _____,
~~sheets~~/fig _____, filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, ~~sheets~~/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE98/00703

V. Resoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-7	YES
	Claims	8	NO
Inventive step (IS)	Claims	1-7	YES
	Claims	8	NO
Industrial applicability (IA)	Claims	1-8	YES
	Claims		NO

2. Citations and explanations

The claimed invention relates to an isolated DNA sequence coding for a mammalian glucuronyl C5-epimerase which converts D-glucuronic acid to L-iduronic acid and a method of producing the enzyme by recombinant DNA-technique.

During the search the following documents were found:

A) The Journal of Biological Chemistry, Patrick Cambell et al, "Biosynthesis of Heparin/Heparan Sulfate", page 26953-26958.

B) WO 9614425

Document A relates to the purified bovine enzyme D-glucuronyl C-5 epimerase. The claimed enzyme has essentially the same characteristics as the known enzyme. However, this isolated enzyme was found to be a truncated form of the enzyme lacking 73 amino acids residues in the N-terminal. Among other residues one of the cysteine residues was missing. In spite of this it was found to be active.

No document, however, has been found relating to an isolated DNA sequence coding for the claimed enzyme or to produce the enzyme by recombinant DNA technique. It is considered inventive to deduce the DNA sequence from the amino acid sequence as the amino acid sequence was not completely known. The new knowledge of the whole amino acid sequence renders it possible to derive the DNA sequence and to produce the enzyme by recombinant DNA technique.

Therefore claims 1-7 are novel and are considered to involve an inventive step.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

That the enzyme is produced by recombinant DNA technique does not automatically render the enzyme novel nor does it automatically give the enzyme an unexpected feature. In this case ,however, because of the fact that the whole amino acid sequence was not known before, the claimed enzyme is novel. Due to the expression "or a functional derivative thereof" of claim 8, this claim cannot, however, be considered to be novel, as this expression would include the enzyme known from document A.

Document B discloses the use of D-glycuronyl-Liduronyl-C5-epimerase enzyme to produce polysaccharides having a high iduronic acid content.

CLAIMS

1. An isolated or recombinant DNA sequence coding for a mammalian, including human, glucuronyl C5-epimerase, or a functional derivative of said DNA sequence, capable of converting D-glucuronic acid (GlcA) to L-iduronic acid (IdoA) constituted by a nucleotide sequence comprising nucleotide residues 1 to 1404, inclusive, as depicted in the sequence listing.

2. A DNA sequence according to claim 1 constituted by a nucleotide residue comprising nucleotide residues 73 to 1404, inclusive, as depicted in the sequence listing.

3. A DNA sequence according to claim 2 constituted by a nucleotide residue comprising nucleotide residues 1 to 1404, inclusive, as depicted in the sequence listing.

4. A recombinant expression vector containing a transcription unit comprising a DNA sequence according to any one of the preceding claims, a transcriptional promoter, and a polyadenylation sequence.

5. A recombinant expression vector according to claim 4, characterized in that the vector is a Baculovirus.

6. A host cell transformed with the recombinant expression vector of claim 4 or 5.

7. A process for the manufacture of a glucuronyl C5-epimerase or a functional derivative thereof capable of converting D-glucuronic acid (GlcA) to L-iduronic acid (IdoA), comprising cultivation of a host cell transformed with a recombinant expression vector according to claim 4 or 5 in a nutrient medium allowing expression and secretion

of said epimerase or functional derivative thereof.

8. A glucuronyl C5-epimerase or a functional derivative thereof whenever prepared by the process of claim 7.

CLAIMS

1. An isolated or recombinant DNA sequence coding for a mammalian, including human, glucuronyl C5-epimerase or a functional derivative thereof capable of converting D-glucuronic acid (GlcA) to L-iduronic acid (IdoA).

2. A DNA sequence according to claim 1 constituted by a nucleotide sequence comprising nucleotide residues 1 to 1404, inclusive, as depicted in the sequence listing.

3. A DNA sequence according to claim 2 constituted by a nucleotide residue comprising nucleotide residues 73 to 1404, inclusive, as depicted in the sequence listing.

4. A DNA sequence according to claim 2 constituted by a nucleotide residue comprising nucleotide residues 1 to 1404, inclusive, as depicted in the sequence listing.

5. A recombinant expression vector containing a transcription unit comprising a DNA sequence according to any one of the preceding claims, a transcriptional promoter, and a polyadenylation sequence.

6. A host cell transformed with the recombinant expression vector of claim 5.

7. A process for the manufacture of a glucuronyl C5-epimerase or a functional derivative thereof capable of converting D-glucuronic acid (GlcA) to L-iduronic acid (IdoA), comprising cultivation of a cell line transformed with a recombinant expression vector according to claim 5 in a nutrient medium allowing expression and secretion of said epimerase or functional derivative thereof.

8. A glucuronyl C5-epimerase or a functional derivative thereof whenever prepared by the process of claim 7.

Replaced by Art 34

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2988293	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
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International Patent Classification (IPC) or national classification and IPC_6 C 12 N 9/90		
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Date of submission of the demand	Date of completion of this report
01.10.1998	30.06.1999
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Telex 17978 PATOREG-S Facsimile No. 08-667 72 88	Authorized officer Yvonne Siösteen/Els Telephone No. 08-782 25 00

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pages _____, filed with the letter of _____.

☒ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. 1-8, filed with the letter of 25.05.1999,
Nos. _____, filed with the letter of _____.

☒ the drawings, ~~sheets~~/fig 1-3, as originally filed,
~~sheets~~/fig _____, filed with the demand
~~sheets~~/fig _____, filed with the letter of _____,
~~sheets~~/fig _____, filed with the letter of _____.

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☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, ~~sheets~~/fig _____

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/SE98/00703

V. Resoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-7	YES
	Claims	8	NO
Inventive step (IS)	Claims	1-7	YES
	Claims	8	NO
Industrial applicability (IA)	Claims	1-8	YES
	Claims		NO

2. Citations and explanations

The claimed invention relates to an isolated DNA sequence coding for a mammalian glucuronyl C5-epimerase which converts D-glucuronic acid to L-iduronic acid and a method of producing the enzyme by recombinant DNA-technique.

During the search the following documents were found:

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No document, however, has been found relating to an isolated DNA sequence coding for the claimed enzyme or to produce the enzyme by recombinant DNA technique. It is considered inventive to deduce the DNA sequence from the amino acid sequence as the amino acid sequence was not completely known. The new knowledge of the whole amino acid sequence renders it possible to derive the DNA sequence and to produce the enzyme by recombinant DNA technique.

Therefore claims 1-7 are novel and are considered to involve an inventive step.

.../...

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

That the enzyme is produced by recombinant DNA technique does not automatically render the enzyme novel nor does it automatically give the enzyme an unexpected feature. In this case ,however, because of the fact that the whole amino acid sequence was not known before, the claimed enzyme is novel. Due to the expression "or a functional derivative thereof" of claim 8, this claim cannot, however, be considered to be novel, as this expression would include the enzyme known from document A.

Document B discloses the use of D-glycuronyl-Liduronyl-C5-epimerase enzyme to produce polysaccharides having a high iduronic acid content.

In the Claims:

Please cancel claims 22-24, 26-32, 36, 37, 44-46, 48-54, 58, 59, 66, 69-71, and 86-102 without prejudice.

Also, please cancel non-elected claims 8, 19 and 20.

Please substitute the following claims 21, 43, 65, 79 and 80 for the pending claims 21, 43, 65, 79 and 80:

21. (Twice amended) An isolated polynucleotide comprising a nucleotide sequence encoding a glucuronyl C5-epimerase capable of converting D-glucuronic acid to L-iduronic acid, the amino acid sequence of which is at least 95% identical to a reference amino acid sequence selected from the group consisting of:

(a) amino acids 25 to 444 of SEQ ID NO: 13 and

(b) amino acids 1 to 444 of SEQ ID NO: 13.

25. The polynucleotide of claim 21 encoding a polypeptide comprising amino acid residues 1-444 of SEQ ID NO: 13.

33. The polynucleotide of claim 21 which is DNA.

34. The polynucleotide of claim 21 which is RNA.

35. The polynucleotide of claim 21, wherein said polynucleotide encodes a polypeptide which is a fusion protein.

38. (Once amended) A vector comprising the polynucleotide of claim 21.

cont

39. The vector of claim 38, wherein said vector comprises a transcription unit.

40.(Once amended) A host cell comprising the polynucleotide of claim 21.

41. The host cell of claim 40, selected from the group consisting of Sf9 cells, *E. coli*, 293 human embryonic kidney cells, COS-1 cells and CHO cells.

42. A method of producing a protein that comprises culturing the host cell of claim 40 under conditions such that said protein is expressed, and recovering said protein.

43. (Thrice amended) An isolated polynucleotide encoding a glucuronyl C5-epimerase capable of converting D-glucuronic acid to L-iduronic acid and which hybridizes under the conditions of incubation at 65° C in a solution comprising: 6x SSC, 5x Denhardt's solution containing 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA, followed by washing in 2x SSC and 0.5% SDS at 42° C, to a polynucleotide encoding a polypeptide selected from the group consisting of:

(a) amino acids 25 to 444 of SEQ ID NO: 13 and

(b) amino acids 1 to 444 of SEQ ID NO: 13.

47. The polynucleotide of claim 43 encoding a polypeptide comprising amino acid residues 1-444 of SEQ ID NO: 13.

55. The polynucleotide of claim 43 which is DNA.

56. The polynucleotide of claim 43 which is RNA.

57. The polynucleotide of claim 43, wherein said polynucleotide encodes a polypeptide which is a fusion protein.

60.(Once amended) A vector comprising the polynucleotide of claim 43.

61. The vector of claim 60, wherein said vector comprises a transcription unit.

62.(Once amended) A host cell comprising the polynucleotide of claim 43.

63. The host cell of claim 62, selected from the group consisting of Sf9 cells, *E. coli*, 293 human embryonic kidney cells, COS-1 cells and CHO cells.

64. A method of producing a protein that comprises culturing the host cell of claim 62 under conditions such that said protein is expressed, and recovering said protein.

65. (Thrice amended) An isolated polynucleotide~~, or an isolated complementary polynucleotide,~~ which encodes a polypeptide having glucuronyl C5-epimerase activity and capable of converting D-glucuronic acid to L-iduronic acid, and which hybridizes under the conditions of incubation at 65° C in a solution comprising: 6x SSC, 5x Denhardt's solution containing 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA, followed by washing in 2x SSC and 0.5% SDS at 42° C, to ~~said isolated~~ a polynucleotide selected from the group consisting of:

(a) nucleotides 73 to 1404 of SEQ ID NO: 12;

(b) nucleotides 73 to 3085 of SEQ ID NO: 12;

(c) nucleotides 145 to 1404 of SEQ ID NO: 12;

(d) nucleotides 145 to 3085 of SEQ ID NO: 12;

(e) nucleotides 1 to 1404 of SEQ ID NO: 12 and

(f) nucleotides 1 to 3085 of SEQ ID NO: 12.

67. The isolated polynucleotide of claim 65 comprising nucleotides 73 to 1404 of SEQ ID NO: 12, or said isolated complementary polynucleotide that hybridizes to the same.

68. The isolated polynucleotide of claim 65 comprising nucleotides 73 to 3085 of SEQ ID NO: 12, or said isolated complementary polynucleotide that hybridizes to the same.

72. The isolated polynucleotide of claim 65 comprising nucleotides 145 to 1404 of SEQ ID NO: 12, or said isolated complementary polynucleotide that hybridizes to the same.

73. The isolated polynucleotide of claim 65 comprising nucleotides 145 to 3085 of SEQ ID NO: 12, or said isolated complementary polynucleotide that hybridizes to the same.

74. The isolated polynucleotide of claim 65 comprising nucleotides 1 to 1404 of SEQ ID NO: 12, or said isolated complementary polynucleotide that hybridizes to the same.

75. The isolated polynucleotide of claim 65 comprising nucleotides 1 to 3085 of SEQ ID NO: 12, or said isolated complementary polynucleotide that hybridizes to the same.

76. The polynucleotide of claim 65 which is DNA.

77. The polynucleotide of claim 65 which is RNA.

78. The polynucleotide of claim 65, wherein said polynucleotide encodes a polypeptide which is a fusion protein.

79. (Twice amended) The polynucleotide of claim 65, wherein said polynucleotide sequence is selected from a member of the group consisting of

(a) nucleotides 73 to 1404 of SEQ ID NO: 12;

(b) nucleotides 73 to 3085 of SEQ ID NO: 12;

(c) nucleotides 145 to 1404 of SEQ ID NO: 12;

(d) nucleotides 145 to 3085 of SEQ ID NO: 12;

(e) nucleotides 1 to 1404 of SEQ ID NO: 12 and

(f) nucleotides 1 to 3085 of SEQ ID NO: 12;

and wherein said polynucleotide encodes a fusion protein.

80. (Thrice amended) ~~A~~ An isolated polynucleotide which encodes an amino acid sequence which has a deletion of the N-terminal, C-terminal or internal regions of the amino acid sequence encoded by the polynucleotide of claim 65, and wherein said polynucleotide sequence is selected from a member of the group consisting of

(a) nucleotides 73 to 1404 of SEQ ID NO: 12;

(b) nucleotides 73 to 3085 of SEQ ID NO: 12;

(c) nucleotides 145 to 1404 of SEQ ID NO: 12;

(d) nucleotides 145 to 3085 of SEQ ID NO: 12;

(e) nucleotides 1 to 1404 of SEQ ID NO: 12 and

(f) nucleotides 1 to 3085 of SEQ ID NO: 12.

81.(Once amended) A vector comprising the polynucleotide of claim 65.

82. The vector of claim 81, wherein said vector comprises a transcription unit.

83.(Once amended) A host cell comprising the polynucleotide of claim 65.

84. The host cell of claim 83, selected from the group consisting of Sf9 cells, *E. coli*, 293 human embryonic kidney cells, COS-1 cells and CHO cells.

85. A method of producing a protein that comprises culturing the host cell of claim 83 under conditions such that said protein is expressed, and recovering said protein.

103.(New) An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide, comprising amino acid residues 1-444 of SEQ ID NO: 13.

NAB 104.(New) The polynucleotide of claim 103 which is DNA.

NAB 105.(New) The polynucleotide of claim 103 which is RNA.

106.(New) The polynucleotide of claim 103, wherein said polynucleotide encodes a polypeptide which is a fusion protein.

107.(once amended) A polynucleotide which encodes an amino acid sequence which has a deletion of the N-terminal, C-terminal or internal regions of the amino acid sequence encoded by the polynucleotide of claim 103 and having glucuronyl C5-epimerase activity and capable of converting D-glucuronic acid to L-iduronic acid.

108.(New) A vector comprising the polynucleotide of claim 103.

109.(New) The vector of claim 108, wherein said vector comprises a transcription unit.

110.(New) A host cell comprising the polynucleotide of claim 103.

111.(New) The host cell of claim 110, selected from the group consisting of Sf9 cells, *E. coli*, 293 human embryonic kidney cells, COS-1 cells and CHO cells.

112.(New) A method of producing a protein that comprises culturing the host cell of claim 110 under conditions such that said protein is expressed, and recovering said protein.

114. An isolated polynucleotide comprising a nucleotide sequence encoding a polypeptide, comprising amino acids 25 to 444 of SEQ ID NO: 13.

115. An isolated polynucleotide, or an isolated complementary polynucleotide, comprising nucleotides 73 to 3085 of SEQ ID NO: 12.